The Global Action Fund for Fungal Infections
Rue de L'Ancien-Port 14
1211 Geneva 1,
Switzerland

www.gaffi.org

Dr Marcus Sprenger
Director
AMR Program
World Health Organisation
Geneva

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Dear Dr Sprenger,

Medical antifungal compounds and their use in agriculture

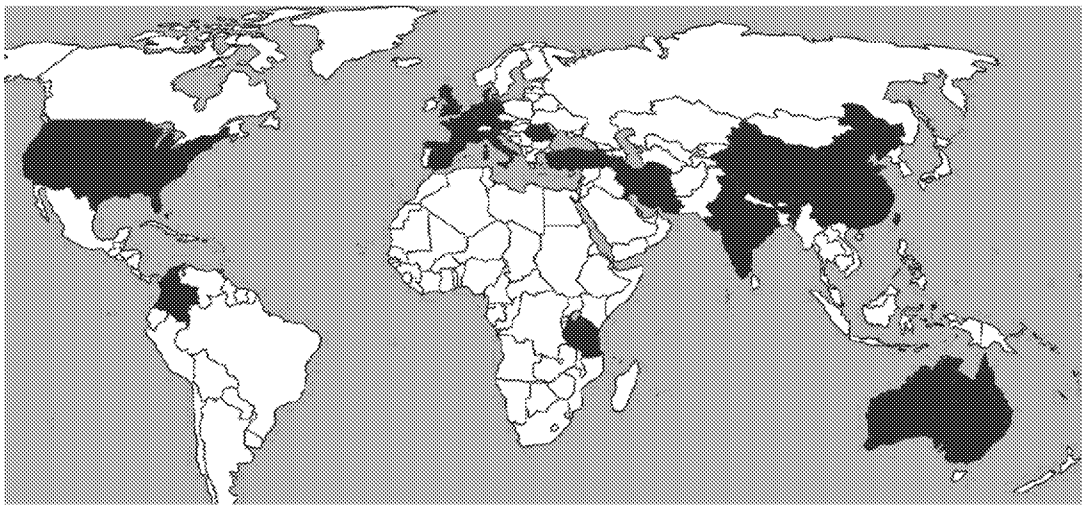
For several decades, we have seen the parallel use of triazole compounds for clinical medicine and as fungicides for crop protection. In medicine, the triazole antifungal drug itraconazole was the first orally active compound for aspergillosis, and numerous other life-threatening fungal diseases, notably the endemic mycoses[1]. Furthermore, other triazole drugs like voriconazole, posaconazole, and the recently launched isavuconazole, are the most clinically effective agents for treatment of invasive aspergillosis, transforming the outcome of leukaemia and transplant patients[2].

In 2007, alarming reports from Manchester and Nijmegen described increasing azole resistance in *Aspergillus fumigatus*, the most important airborne mould pathogen and allergen worldwide[3]. Broadly, two circumstances lead to the growing resistance problem. Firstly, in the environment, strains highly resistant to azole and triazoles emerged rapidly as a consequence of the widespread use of azole fungicides. Such strains are characterised by two principle genetic signatures (TR_{34}/L98H and TR_{46}/Y121F/T289A). Secondly, in patients on long term therapy, strains acquire a variety of resistance mechanisms including target site mutations, increased target copy number, efflux and other mechanisms still being described. Around 90% of all individuals with resistant infections in the context of invasive aspergillosis will die, and those with chronic and allergic aspergillosis become untreatable. Fortunately person-to-person transmission of *A. fumigatus* is barely described despite the fact that all infections are acquired through inhalation.

Environmentally derived resistant strains of *A. fumigatus* remain the greatest global threat to human health. The TR_{34}/L98H and TR_{46}/Y121F/T289A mutations have been repeatedly documented in isolates in the environment from numerous countries covering five continents. This is shown in the map below[4] and described in detail in an international study, published in 2015[5]. *Aspergilli* are ubiquitous and easily spread by air currents and as fomites. In some areas resistance is reaching levels that have prompted some centres to move away from azoles as a sole first line therapeutic, with high cost combination and/or less effective agents being used. Of note, in many countries the resistance

frequency is unknown, as susceptibility testing of moulds is not routinely performed.

Figure. Countries in which azole resistance in *A. fumigatus* has been described.



The greatest concern is the worsening of the current situation, as new azole resistance mutations continue to emerge in the environment; most recently TR_{98}/Y121F/T289A and TR_{138}/Y121F/T289A mutations have been found in environmental and clinical samples in the Netherlands. If action is not taken there is a realistic probability that the azole class will be lost for clinical use.

There is a substantial body of circumstantial evidence that the use of five azole fungicides is primarily responsible for this problem, at least in Europe. Much of this data is summarised in the European Centres for Disease Control report (2013)[6], and supported by subsequent studies from individual countries[5,7,8]. Agricultural fungicide remains the primary driver of resistance, although the careless disposal of bioactive synthesis intermediates of azoles could contribute to the problem. Detection of azoles in water and other environments is well documented.

To help protect medically-important triazoles, a reduction of usage of these triazole fungicides is necessary. There are multiple uses of fungicides which do not contribute to food sustainability and independence, including the flower and bulb/seed industry, fencing and gypsum plasterboard, antifungal preparations for consumers wanting to eradicate fungi from households and luxury crops, such as soft fruits and vines. A phased withdrawal from these markets would be welcome, and would reduce AMR pressure and specifically the development and spread of multi-azole and pan-azole resistance.

To help retain the class of medically-important azoles immediate action is required. Key is a reduction of usage of these triazole fungicides. Additionally, the following steps should be considered:

1. To prove the relation between azole fungicides use and resistance selection in *A. fumigatus* and investigate which factors are critical to resistance selection. It is highly likely that the application of fungicides

and management of composting of fungicide exposed waste are important drivers of resistance selection in *A. fumigatus*, effectively collateral damage of major importance for human health. Insights in these factors will allow evidence-based strategies aimed at substitution of azoles by other compounds or changes to waste management that reduce the risk for new mutations to emerge and/or the spread of existing mutations.

2. To set up an international network for surveillance of resistance to antifungal drugs for the main human fungal pathogens in designed Reference Laboratories across the world (human and environmental strains).
3. As *A. fumigatus* is not a plant pathogen, the emergence of resistance is an unintended side effect of the agricultural application of fungicides. The authorization procedure for new fungicides should include testing for activity against non-target fungi such as *A. fumigatus,* that are known to cause infections in humans.
4. New antifungal drugs with novel chemistries and modes of action in clinical development and/or commercially launched after regulatory approval should never be used as fungicides in agriculture.

As the December 2015 O'Neill report (Antimicrobials in Agriculture and the Environment: Reducing unnecessary use and waste)[9] articulates so clearly, it is important that future novel antifungals do not follow the same path of dual medical and agricultural use. If the WHO and other national and international agencies were able to reserve such new compounds exclusively for use, this would minimise the likelihood of resistance in environmental isolates of all fungi, but notably *A. fumigatus*. Even new compounds without apparent activity against *Aspergilli* should be included within this restriction, as later chemical analogues, or combination antifungal therapy could be of benefit for human health. The number of patients potentially affected is shown in the table below (annual incidence for invasive aspergillosis, prevalence for chronic and allergic disease)[10].

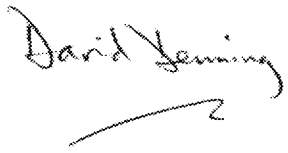
Aspergillosis	Invasive	Chronic	Allergic
Global Burden	200,000 – 400,000	1.5 M – 3M	6M – 20M
Untreated Mortality	~ 100%	~ 75% / 5 yrs	<1%*
Treated Mortality	30-85%	~ 45% / 5 yrs	<1%
Orphan Drug Designation?	Yes	Yes	No (possible for Cystic Fibrosis)
Responsive to azole antifungals	Yes – optimal therapy	Yes, only long term therapy option	Yes, only long term oral therapy option

* Severe asthma is responsible for 400,000+ deaths annually, and many probably have fungal sensitisation and could respond to antifungal therapy.

There are a number of novel antifungals in clinical development currently, including F901318, Nikkomycin Z, SCY-078, VT-1161, and several others in preclinical development, including ASP2397, BHBM, ASP9726, Biafungin, VT-1129, Ilicicolin H, L365, L743 and L884[11]. Therefore these considerations are not purely theoretical and need immediate action.

We applaud your efforts to control antimicrobial, including antifungal, resistance. We (and I write on behalf of multiple colleagues focused on this problem across the world) appreciate that is not a simple task, but minimizing current azole resistance frequency and spread and avoidance of future resistance problems would be most welcome.

Sincerely yours,



David W Denning
President, Global Action Fund for Fungal infections
Professor of Infectious Diseases in Global Health
Director, National Aspergillosis Centre

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